

EXHIBIT C

13. S. I. Rasool and S. H. Schneider, *Science* 173, 138 (1971); G. Yamamoto and M. Tanaka, *J. Atmos. Sci.* 29, 1405 (1972).
14. J. Namias, in *The Changing Chemistry of the Oceans*, D. Dyrssen and D. Jagner, Eds. (Wiley, New York, 1972), p. 27.
15. S. Martin and P. Kauffman, *J. Fluid Mech.* 64, 507 (1974); P. K. Weyl, *Meteorol. Monogr.* 8 (No. 30), 37 (1968).
16. E. N. Lorenz, *J. Appl. Meteorol.* 9, 325 (1970); in *Man's Impact on the Climate*, W. H. Mathews, W. W. Kellogg, G. D. Robinson, Eds. (MIT Press, Cambridge, Mass., 1970), p. 179; *Meteorol. Monogr.* 8 (No. 30), 1 (1968).
17. C. E. Leith, *J. Appl. Meteorol.* 12, 1066 (1973); R. M. Chervin, W. L. Gates, S. H. Schneider, *J. Atmos. Sci.*, in press.
18. J. Smagorinsky, in *Weather and Climate Modification*, W. N. Hess, Ed. (Wiley, New York, 1974), p. 633.
19. S. Manabe and R. T. Wetherald, *J. Atmos. Sci.* 24, 241 (1967).
20. M. I. Budyko, *Tellus* 21, 611 (1969).
21. ———, *Eos Trans. Am. Geophys. Union* 53, 868 (1972).
22. W. D. Sellers, *J. Appl. Meteorol.* 8, 392 (1969); *ibid.* 12, 241 (1973).
23. S. H. Schneider and T. Gal-Chen, *J. Geophys. Res.* 78, 6182 (1973).
24. L. Machta, in *Carbon and the Biosphere*, G. M. Woodwell and E. V. Pecan, Eds. (USAEC CONF-720510, National Technical Information Service, Springfield, Va., 1973), p. 21.
25. E. A. Martell, in *Man's Impact on the Climate*, W. H. Mathews, W. W. Kellogg, G. D. Robinson, Eds. (MIT Press, Cambridge, Mass., 1971), p. 421.
26. W. E. Cobb, *J. Atmos. Sci.* 30, 101 (1973); L. Machta and K. Telegadas, in *Weather and Climate Modification*, W. N. Hess, Ed. (Wiley, New York, 1974), p. 687.
27. R. A. Bryson, *Weatherwise* 21, 56 (1968); *Climatic Modification by Air Pollution, II: The Sahelian Effect* (Institute for Environmental Studies, University of Wisconsin, Madison, 1973); *Science* 184, 753 (1974).
28. J. M. Mitchell, Jr., in *Man's Impact on the Climate*, W. H. Mathews, W. W. Kellogg, G. D. Robinson, Eds. (MIT Press, Cambridge, Mass., 1971), pp. 133 and 167.
29. P. V. Hobbs, H. Harrison, E. Robinson, *Science* 183, 909 (1974).
30. W. W. Kellogg, in *The Energy Question*, E. W. Erickson and L. Waverman, Eds. (Univ. of Toronto Press, Toronto, 1974), p. 242.
31. A. M. Weinberg and R. D. Hammond, *Am. Sci.* 58, 412 (1970).
32. Club of Rome, *Ecologist* 2, 1 (1972); R. L. Heilbroner, *An Inquiry into the Human Prospect* (Norton, New York, 1974).
33. J. O. Fletcher, *The Heat Budget of the Arctic Basin and Its Relation to Climate* (Report R-444-PR, Rand Corporation, Santa Monica, Calif., 1965).
34. M. Warshaw and R. R. Rapp, *J. Appl. Meteorol.* 12, 43 (1973).
35. G. A. Maykut and N. Untersteiner, *J. Geophys. Res.* 76, 1550 (1971).
36. SCEP report, *Man's Impact on the Global Environment: Study of Critical Environmental Problems* (MIT Press, Cambridge, Mass., 1970); C. E. Leith, Ed., *The Natural and Radiatively Perturbed Troposphere* (CIAP Monograph 4, Department of Transportation, Washington, D.C., in press).
37. S. H. Schneider, paper presented at the Annual Meeting of the AAAS, December 1972, Washington, D.C.; *New Eng.* 7, 14 (1973).
38. B. C. Farhar, *Bull. Am. Meteorol. Soc.* 55, 759 (1974).
39. L. A. Mayer, *Fortune* 84 (No. 2), 85 (1974).
40. S. H. Schneider, *National Observer*, 6 July (1974), p. 18; see also R. F. Taubenfeld and H. J. Taubenfeld, *International Organization* 23, 808 (1969).
41. ———, *Ambio* 3, 150 (1974). In the same issue of *Ambio* (p. 120) M. Falkenmark and G. Lindh suggest: "It would, for instance, be of interest to suppress part of the copious rains in Cameroon, Nigeria, and other coastal states to keep more moisture in the monsoon for condensation further inland." Such plans are not new. For example, G. A. Thompson proposed "A plan for converting the Sahara Desert into a sea" in the 10 August 1912 issue of *Scientific American* (p. 114). Obviously, more schemes will be advanced.
42. H. Landsford, *Bull. Am. Meteorol. Soc.* 54, 658 (1973).
43. W. D. Sellers, private communication.
44. E. LeRoy Ladurie, *Histoire du Climat depuis l'An Mil* (Flammarion, Paris, 1967).
45. We wish to acknowledge the encouragement of G. Kukla, organizer of the AAAS symposium "To Feed the World" (San Francisco, 27 February 1974), at which this paper was originally presented. The National Center for Atmospheric Research is sponsored by the National Science Foundation.

Viral Infection and Host Defense

Many aspects of viral infection and recovery can be explained by the modulatory role of double-stranded RNA.

William A. Carter and Erik De Clercq

Current interest in double-stranded RNA's (dsRNA's) takes on many forms. It ranges from physicochemical studies of their structure, through descriptions of the large diversity of cellular reactions brought about by these molecules, to studies of events triggered at the level of the intact animal.

We attempt in this article to develop a perspective on the heterogeneity of reactions provoked by dsRNA in biological systems. We describe how chemical lesions (bond breakage, unpaired

bases) in the double-helical structure can modulate or abort biological function. Finally, we submit for consideration a hypothesis that dsRNA is both the molecular mediator of much of the morbidity and cellular damage associated with cytolytic viral infection, as well as a crucial molecular trigger that stimulates many of the organism's defenses to viral infection. By defining this dynamic role of dsRNA, we hope to signal new experimental inquiry which may permit a more detailed analysis of events at the molecular level, which until now have been described at the microscopic level as "extreme tissue damage probably due to a virus."

Before we proceed with development of ideas on the role of dsRNA in viral

infection, it should be recalled that dsRNA is generally considered as not being a regular constituent of the eukaryotic cell. This view is clearly correct in a quantitative sense, although it may require some revision. For example, it has been shown that heterogeneous nuclear RNA contains double-stranded regions (1). Recently, dsRNA from nuclei of HeLa cells has been isolated (2) and shown to have molecular weight in excess of ~25,000. It is postulated that dsRNA may interact with an initiation factor thought to be necessary for messenger RNA (mRNA) translation (3); a helical region greater than 20 base pairs seems to be involved in this recognition. The amount of dsRNA in ascites tumor cells appears to be under control of a specific nuclease (4), and thus the extent and the rate of translation could be regulated by this mechanism. Such evidence supports the view that dsRNA may have a regulatory role in protein synthesis within mammalian cells.

Interferon Induction by dsRNA

Many specialized cellular functions are altered in cells exposed to dsRNA. One of the most characteristic functions triggered by dsRNA is the production of interferon. Various dsRNA's of both biological and synthetic origin have been shown to stimulate interferon production:

Dr. Carter is chairman of the Department of Medical Viral Oncology, Roswell Park Memorial Institute, and is a professor and chairman of the Graduate Faculty in Microbiology, State University of New York at Buffalo, Roswell Park Division, Buffalo 14203. Dr. De Clercq is a member of the faculty of the Katholieke Universiteit te Leuven, Rega Institute, Minderbroedersstraat 10, B-3000, Leuven, Belgium.

1) Double-stranded RNA's of animal viruses [for example, reovirus (5)], insect viruses [for example, cytoplasmic polyhedrosis virus (6)], plant viruses [for example, rice dwarf virus (6) and wound tumor virus (7)], mold viruses [mycophages of *Penicillium funiculosum* (8), *P. stoloniferum* (8, 9), *P. cyaneofulvum* (10), *P. chrysogenum* (11), and *Aspergillus niger* (12)], and bacterial viruses [bacteriophages such as replicative forms of MS 2 (13) and MU 9, and f2 (14) coliphages] are potent interferon inducers.

2) Double-stranded RNA with interferon-inducing (or virus-inhibitory) activity has also been demonstrated in "normal," ostensibly uninfected, mammalian cells such as chick embryo, chick liver, rat liver, and rabbit kidney, and in HeLa cells (15). This dsRNA represents about 0.01 percent of the total RNA of the cell (15) [newer estimates suggest as much as 0.1 to 1 percent (17)]. Its general occurrence in cells of different animal species and the finding that it can be hybridized with host cell DNA (15) argue against a viral origin of these RNA's. Whether its biosynthesis is sensitive or resistant to actinomycin D remains controversial (15).

3) The DNA viruses and single-stranded RNA (ssRNA) viruses are also potent interferon inducers. It is not unequivocally established, however, whether their interferon-inducing capacities result from the input nucleic acids (DNA or ssRNA), or traces of dsRNA in the input virions, or intermediary dsRNA formed during the viral replicative cycle within infected cells. Evidence for the last alternative is perhaps strongest and has been provided with both RNA [mengo (18), influenza (15)] and DNA [vaccinia (15)] viruses; it should be noticed, however, that interferon induction does not appear to correlate exclusively with dsRNA content or synthesis (16). Some late events in viral replication may also be mandatory for induction.

4) Synthetic dsRNA's: Homopolymer pairs (19) such as $rI_n \cdot rC_n$, $rA_n \cdot rU_n$, and $rG_n \cdot rC_n$, and copolymers such as $r(I,C)_n$, $r(A,U)_n$, and $r(G,C)_n$, [as reviewed recently (20)].

In contrast with dsRNA, ssRNA's, ds- and ssDNA's, and DNA-RNA hybrids are all rather ineffective interferon inducers (21). Occasionally, interferon-inducing activities have been described with synthetic ssRNA's, especially when they form complexes with

basic substances such as DEAE-dextran, methylated albumin, protamine, or neomycin (22). The interferon titers obtained with these ssRNA's are considerably lower than those obtained with dsRNA. In general, dsRNA's are 10^3 -fold to 10^5 -fold more active in interferon induction than any complexes containing RNA in a hybrid or single-stranded form. This difference in activity, of several orders of magnitude, provides a ground for our first assumption—namely, that only RNA's with double helices will be quantitatively important in triggering antiviral responses. As we will see, this is a most reasonable assumption, since the extent of triggering the interferon receptor can be a quantitative indicator of double-helical content (23).

The reactivity of cells, in terms of interferon induction by dsRNA, has permitted a type of mapping study in which one titrates the tolerance of the receptor (presumably, membrane located) to fine lesions in the degree of secondary structure of the inducer complex. For example, the structural requirements of the receptor on human fibroblasts have been studied (23); in this work, the rI_n or rC_n strand in the complex has been interrupted either by unpaired bases (U or G) or by bond breakage. The complexes $r(I_{99},U)_n \cdot rC_n$ and $r(I_{21},U)_n \cdot rC_n$ have little induction ability, while $rI_n \cdot r(C_{20},G)_n$, $rI_n \cdot r(C_{22},U)_n$, $rI_n \cdot r(C_{13},U)_n$, and $rI_n \cdot r(C_7,U)_n$ are active. Similarly, complexes of rI_n and oligocytidylylate [such as $rI_n \cdot (Cp)_{23}G > p$] are active, while complexes of oligoinosinate and rC_n (such as $rI_{10} \cdot rC_n$) are generally inert. Thus, for interferon induction, structural requirements in strand continuity and base pairing are apparently much more stringent in the rI_n strand than in the rC_n strand. An important aspect of this work has been the observations that two inducers, $rI_n \cdot r(C_{13},U)_n$ and $rI_n \cdot r(C_{20},G)_n$, may have better therapeutic indexes than $rI_n \cdot rC_n$ itself. They are hydrolyzed by nucleases up to eightfold faster, and yet are nearly as active as unmodified $rI_n \cdot rC_n$ (23). These observations form a ground for our second assumption; namely, that a particular biological effect—for example, interferon-inducing ability—depends not only on double strandedness of the inducer, but also on fine structural features of one of the two strands. We project that a "fine structure" term can be sought and developed for other reactions triggered by dsRNA's in biological systems. If other events trig-

gered by dsRNA's also depend on "fine structure" and not only on double-helical conformation, then one can visualize a basis for the diversity of biological effects of the ostensibly uniform family of dsRNA's.

Intrinsic "Toxicity" of dsRNA

Double-stranded RNA triggers many events in addition to the receptor for interferon induction. Another conspicuous event is the inhibition of protein synthesis, both in cell extracts (4, 24, 25) and intact cell cultures (26). The shutoff of host protein synthesis, generally observed in cells infected with lytic viruses (for example, poliovirus), seems related to formation of intermediary dsRNA. The rate of appearance of dsRNA in the infected cell parallels the decline in rate of cellular protein synthesis (26). The accumulation of dsRNA in mammalian cells infected with lytic viruses is not only associated with a decline in host cell protein synthesis, but, eventually, with death of the cell (27). In yeast cells, cell-killing has also been associated with dsRNA (28), and the production of dsRNA has been incriminated as well as a molecular mediator of the destructive action of some viruses on tumor cells in mice (27, 29).

In addition to effects on protein synthesis, dsRNA has a specific effect on DNA synthesis and, therefore, on cell division. For example, $rI_n \cdot rC_n$ inhibits DNA synthesis (stimulated by isoproterenol) in salivary gland cells and mitosis in liver cells (stimulated by partial hepatectomy) (30).

In the whole animal, various adverse effects have been observed with dsRNA, especially in those species (mice, rats, rabbits) that are most responsive to the interferon-inducing capacity of dsRNA. These effects include a local Schwartzman phenomenon, pyrogenicity, embryotoxicity (fetal resorption), and ocular toxicity (lens opacification, iris hyperaemia, and aqueous flare) in the rabbit (31). In mice and rats, a diminution of hemopoietic stem cells in bone marrow and spleen (and of peripheral white blood cells), as well as induction of a runtlike disease (thymic atrophy, spleen hypoplasia), has been noted (32). An accelerated onset of malignant tumors and autoimmune disease has also been reported (33). Finally, cellular toxicity (edema and hemorrhage due to destruction of endothelial

cells of the small blood vessel) occurs in the chicken (34).

A consistent toxic manifestation observed in man upon administration of relatively low doses of dsRNA ($rI_n \cdot rC_n$) has been fever (35). Production of fever is probably related to stimulation of release of a granulocyte-specific polypeptide, pyrogen. This product, in turn, effects a rise in body temperature by exerting its effect on the hypothalamic thermal regulatory "center." For this discussion, it is important to note that fever production is, for all intents, only observed with one class of nucleic acid molecules—namely, dsRNA's. DNA-RNA hybrids and ssRNA are 10^2 -fold to 10^3 -fold less pyrogenic, which suggests that these molecules probably never cause thermoregulatory problems in vivo.

The question arises as to what is the molecular basis for each of these toxic effects. Although multiple loci of inhibition of cellular function could be operative, we favor a simple model in which most of these toxicities would be achieved by inhibition of protein and DNA synthesis, or both.

Different possibilities have been envisaged to explain the mechanism of inhibition of protein synthesis by dsRNA. Most recently, Kaempfer and Kaufman (25) attributed the inhibitory activity of dsRNA on protein synthesis to an inactivation of IF-3, an initiation factor required for the recycling of ribosomes and for their binding to mRNA. The concentrations of dsRNA necessary to achieve strong inhibition of IF-3 are roughly comparable to those necessary to stimulate extracellular interferon production, although they are 10^3 to 10^5 times higher than those necessary to achieve intracellular antiviral protection (which is probably conferred simply by the intracellular molarity of interferon).

An inspection of the relative sensitivities of two events—interferon induction and inhibition of cellular protein synthesis—to concentrations of dsRNA might bear on understanding why the intracellular molarity of dsRNA does not always elicit the expected interferon level. Interferon production seems to require *de novo* protein synthesis (36), so that if the intracellular concentration of dsRNA rose too quickly, the protein machinery would be operationally closed and unable to accommodate incoming mRNA's.

We submit that the concentration

of dsRNA could serve as a type of chemostat that modulates the background of residual cellular protein synthesis. It seems likely that the chemostat for triggering the interferon receptor is set somewhat below that necessary to activate the inhibition of IF-3. Obviously, compartmental considerations (such as the location of the dsRNA receptor relative to the functional polysomal machinery), as well as kinetic ones, may turn out to be major terms in modulating the interferon concentration finally achieved. These considerations would explain, of course, why high interferon concentrations are not always found where they are expected, simply on the basis of the recoverable amount of dsRNA. We can restate these ideas in the form of two "equations." (i) Interferon concentration is equal to k_1 times the concentration of dsRNA times the time, and (ii) the percentage of inhibition of protein synthesis is equal to k_2 times the concentration of dsRNA times the time. In both cases, the time term implies that period in which double-helical structure is preserved. Probably a "compartment" term is also necessary, to cover concentration gradients that would be expected to exist within the cell, as well as across the plasma membrane on which the interferon trigger receptor is presumably located. Our fine structure term might also be important here, but further studies are needed to define the reactivity of IF-3 to dsRNA's with varying degrees of fidelity in total base pairing. It may be a sufficient treatment to suggest that a dynamic situation could exist in which receptors compete for dsRNA molecules and that the outcome of these "races" in each cell (multiplied by, say, the 10^9 to 10^{12} cells per tissue bed) could have important implications for the whole animal.

One particular phenomenon evoked by dsRNA, namely, fever production, deserves further comment. Evidence now suggests that the efficiency of replication of certain viruses, particularly those with an upper respiratory tropism, can be modulated by small changes in temperature (as discussed later). Thus, we wonder if fever production has not evolved as a specific response to the presence of dsRNA molecules in the circulation: the availability of dsRNA molecules would be facilitated by tissue destruction and leakage of nucleic acid from

cells during the packaging steps associated with late events in the viral replication cycle. It has been interesting to note that fever production in the rabbit, triggered by dsRNA's, is exquisitely sensitive to the fidelity in base pairing of the input polymer pair (37). For example, infrequent "loops" (one nucleotide per 20 base pairs) in an otherwise perfectly helical $rI_n \cdot rC_n$ molecule [such as $rI_n \cdot r(C_{18}U)_n$] dramatically changes its fever production properties. Its activity as an interferon inducer remains constant despite this modification.

The foregoing experiments emphasize an important conceptual point. Biological effects of dsRNA's, like fever production, may vary in magnitude not only in response to the serum molarity of dsRNA, but also in response to subtle conformational features. At the present time, we do not know whether the ability to trigger one reaction, without the other, is due to specific structure-function considerations or simply to kinetic considerations. For example, it might take a significantly longer lag time to trigger fever responses than to trigger interferon production. The interferon trigger is known to respond quickly—in cell culture it requires less than 5 minutes (38). Since introduction of "loops" facilitates nucleolytic attack, trigger or nontrigger decisions may just depend on the half-life of the dsRNA, rather than on the structural configuration per se.

Double-Stranded RNA in Acute Infections

Infection with highly cytotoxic viruses (for example, picornaviruses) leads to rapid destruction of the cell. Amako and Dales (39), as well as Haase *et al.* (40), attributed the virus-induced (mengo) cell death to a viral protein synthesized at the end of its replicative cycle. However, this view is contested by Cordell-Stewart and Taylor (27), who have reported rapid destruction of the same virus-infected cells in the absence of viral protein synthesis since, in their restrictive cells, only dsRNA was synthesized. If we assume that a viral protein synthesized late in infection is indeed responsible for the cytopathology, it is hard to understand how interferon would fail to prevent cell death in the mengovirus-infected cell (40). We would antici-

pate that such viral protein (or proteins) would not be synthesized in the interferon-treated cell (41); this evidence may be, therefore, indirect support that dsRNA is a better candidate to explain this particular cytopathic effect. As we have stated, dsRNA is formed as an intermediate in the replication cycle of most, if not all, cytopathic viruses (42), and it generally accumulates throughout the infection cycle. Eventually, it may be a major viral RNA species at the time of cell death. Clearly, implication of dsRNA as a mediator of local and systemic toxicity would not restrict the roles of other virus-induced products in the process of evolving the total pathologic picture. For example, other products might "polish" or create a unique pathologic situation by modifying an organism or tissue already acted upon by dsRNA.

Does the dsRNA molecule play a major role in the toxicity evoked by most animal viruses? To incriminate dsRNA as a principal causative agent of the cytopathic effect of viruses and the clinical symptoms of certain viral diseases, some requirements would have to be met. First, the dsRNA should indeed be produced during the multiplication of most, if not all, cytopathic viruses. Second, the pathologic manifestations of virus infections should mimic those directly obtained with administration of dsRNA. And, third, the dsRNA should be made in sufficiently high amounts during the infection to account for both the general and local toxic manifestations of the disease. Evidence is rapidly emerging to suggest that these requirements are fulfilled.

1) Formation of a double-stranded, replicative intermediate is a general characteristic of the multiplication of RNA viruses. Recently, dsRNA has also been demonstrated in cells infected with DNA viruses such as vaccinia virus (15, 43) and adenovirus (17, 44). It has not yet been shown to occur in cells infected with herpes simplex virus, although possibly the critical experiments have not been performed. Moreover, herpes simplex infections—in contrast to vaccinia and adenovirus infections—are commonly asymptomatic and produce only local lesions (for example, "fever" blisters), usually upon exposure of the host to some provocative stimuli (menstruation or fever). If dsRNA is a major mediator of the systemic toxicities oc-

curing in viral infections, then we hypothesize that dsRNA may appear in relatively low quantities in the herpes-infected tissue.

2) Both the local and general manifestations of viral infection are reminiscent of the toxic effects that we have previously noted with dsRNA. In cells, there is damage (cloudy swelling, vacuolation) and death (margination of nuclear chromatin, pyknosis); and, in whole organisms, there is fever, chills, malaise, headache, and prostration. Some virus infections may remain localized to the portal of entry (influenza and other respiratory tract infections), whereas others spread through the organism and eventually settle in the so-called target organs (such as skin, central nervous system, and liver). Local or systemic spread is probably determined in part by specific affinities of viruses for certain cells. Our hypothesis, posing dsRNA as a molecular mediator of local and systemic toxicity, would accommodate a lingering dilemma in the pathogenesis of certain viral infections. Why, for example, do infections with adenovirus, influenza, and rhinovirus cause profound systemic effects (like prostration or fever) in the absence of virions ever being detected at any distance from the original (local) target tissue? The availability of dsRNA molecules in the original tissue, its biological reactivity, and its stability in biological fluids suggest that dsRNA is a reasonable candidate to mediate the systemic effects.

3) It seems to us that the dsRNA may be produced in sufficiently high quantities to account for the wide array of general and local toxic manifestations seen in the virus-infected host. We have commented on the specific activity of dsRNA, particularly in terms of its ability to induce generalized toxic effects at extremely low doses (for example, pyrogenicity in the rabbit is sustained for 20 to 40 hours with as little as 1 microgram per kilogram of body weight) (32, 37). It seems reasonable to assume that equivalent amounts of dsRNA may be made during virus infections, and released into the blood stream upon lysis of the infected cell, although as yet we lack any direct concentration measurements. Techniques are now available for quantitative recoveries of small concentrations of dsRNA existing in a large pool of macromolecular nucleic acid (45). Thus, it should now be possible,

for example, to percolate human serum through a CF-11 (Franklin) column and thereby determine the molarity of the RNA species present as a by-product, say, of influenza infection.

In addition, we point out that the interferon produced during the replicative cycle of the virus probably will potentiate the sensitivity of the uninfected host cell to the toxicity of the newly synthesized dsRNA. Such increased toxicity of dsRNA in interferon-treated cells has been clearly demonstrated in cell culture (46) and may also apply to viral infection in the whole animal (47). Only dsRNA's show an enhanced toxicity in the interferon-treated cell; various other substances tested—including ssRNA's, dsDNA's, and metabolic inhibitors—do not prove more toxic in interferon-treated than in untreated cells (48). This interesting phenomenon could, in fact, increase the effects of dsRNA by up to one order of magnitude. We suggest that this phenomenon presents a biological paradox: namely, interferon produced as a defensive response renders the uninfected cell actually more susceptible to the toxic effects of dsRNA molecules released by the infected cell.

Host Defense Mechanism

Another facet in the wide spectrum of biologic activities displayed by dsRNA's is their adjuvant effect on both humoral and cellular immunity. In mice, this effect can be shown with a variety of antigens, including sheep red blood cells and transplantation antigens (49). Double-stranded RNA acts as a potent mitogen for human peripheral blood leukocyte cultures, as well as for mouse spleen and bone marrow cells *in vitro* (50). *In vivo*, either enhancement or depression of the immune response can be observed to depend on several factors, including the time of RNA injection in relation to antigen delivery and time of actual measurement of the immune response (49). The transient immunosuppression noted "early" (on days 3, 4, and 5 after antigen injection) has been attributed to a cytopathic action of dsRNA on the reticuloendothelial cell (49). The stimulatory effect on dsRNA on antibody production (frequently seen on days 7 and 8 after antigen injection) has been ascribed to an absolute increase in the number

Table 1. Classification of animal virus genetic systems. This table is modified from data in (60).

Class	Genome replication	Viral genome integration with host*	Messenger RNA transcription†	Virion-associated polymerases for mRNA synthesis	Examples
1	$\pm\text{DNA} \rightarrow \text{DNA}$	+(P)	$+\text{DNA} \rightarrow +\text{RNA}$	Yes (pox)	DNA viruses (that is, herpes, adeno-, pox-, polyoma viruses)
2	$+\text{RNA} \rightarrow \begin{cases} -\text{RNA} \\ +\text{RNA} \end{cases} \rightarrow \pm\text{RNA}$	+(M)	$\pm\text{DNA} \rightarrow +\text{RNA}$	No	Oncornaviruses
3	$+\text{RNA} \rightarrow \pm\text{RNA} \rightarrow +\text{RNA}$		$\pm\text{RNA} \rightarrow +\text{RNA}$	No	Picornaviruses, (?) arboviruses
4	$-\text{RNA} \rightarrow \pm\text{RNA} \rightarrow \pm\text{RNA}$		$-\text{RNA} \rightarrow +\text{RNA}$	Yes	Rhabdoviruses, (?) myxo- and paramyxoviruses
5	$\pm\text{RNA} \rightarrow \pm\text{RNA}$		$\pm\text{RNA} \rightarrow +\text{RNA}$	Yes	Reovirus

* (P), possible route; (M), mandatory route.

† Messenger RNA is defined as the plus (+) strand in all systems.

of antibody producing cells. The molecular basis of these enhancement and suppressive effects on the immune systems are not known at present. Possibly the effects on growth of malignant tumors and triggering of autoimmune disease (as has been discussed in connection with the intrinsic toxicity of dsRNA) are related to similar molecular events.

These effects may be important in the recovery from acute viral infection. With certain viral infections, like measles, serum interferon appears first, and specific immunoglobulin appears later (51). The load of serum dsRNA, by acting on the immunocompetent cell, might serve to dampen, or heighten, the immune responsiveness. Thus, it could accelerate or postpone the detection of specific antibody, and thereby modulate the rate of recovery from the morbid condition. That bone marrow-derived (B) cells and thymus-derived (T) cells may

both be primary targets for the effects of dsRNA on the immune response is indicated by the recent experiments of Cone and Johnson and Jaroslow and Ortiz-Ortiz (52); these experiments suggest that dsRNA (such as $\text{rA}_n \cdot \text{rU}_n$) appears to preferentially act on the thymus-derived cell.

Recovery from Acute Virus Infection

In view of its diverse biologic activities documented above, we suggest that dsRNA produced during an acute viral infection (Table 1) will play a role, perhaps an important one, in the recovery from disease. It may be useful to review the levels at which these molecules could exert effects, and to consider the collective responses of the organism carrying concentrations of dsRNA in specific and generalized body compartments (Fig. 1).

1) Interferon induction. Interferon

blocks virus replication (probably at the translational level) and, in addition, exerts a variety of nonantiviral effects. These include an enhancement of the phagocytic activity of macrophages (53) and stimulation of the immunolytic activity of lymphocytes (54). These "secondary" effects of interferon may promote recovery from the virus infection by serving to operationally localize the infection.

2) Stimulation of the production of virus specific antibodies (immunoglobulins). These immunoglobulins should assist in curtailment of infection (and, especially, reinfection) by neutralizing the virus either locally (at the mucosae, for example, IgA) or systematically, as in the blood (IgG, IgM). The concentration of dsRNA may prove to be especially important since, as we have seen, actual blunting of the immune response can occur under certain experimental conditions.

3) Enhancement of the cell-mediated immune response (delayed hypersensitivity). Accumulating evidence suggests that, at both the portal of entry and at the target organ, cellular immune responses are important in the recovery from virus infection (55). Cellular immunity appears to operate, in part, through the production of lymphokines (56); these are effector molecules generated during the interaction of sensitized lymphocytes with specific antigens. However, they may also be induced nonspecifically in the unsensitized cell exposed to dsRNA. Various lymphokines have been described and include lymphocyte-transforming factors, macrophage inhibiting and activating factors, skin reactive factors, chemotactic factors, cytotoxic factors, and interferon-like factors. Thymus-derived lymphocytes are generally regarded as the major source of lymphokines (57), although lymphokines may also be formed by other cell

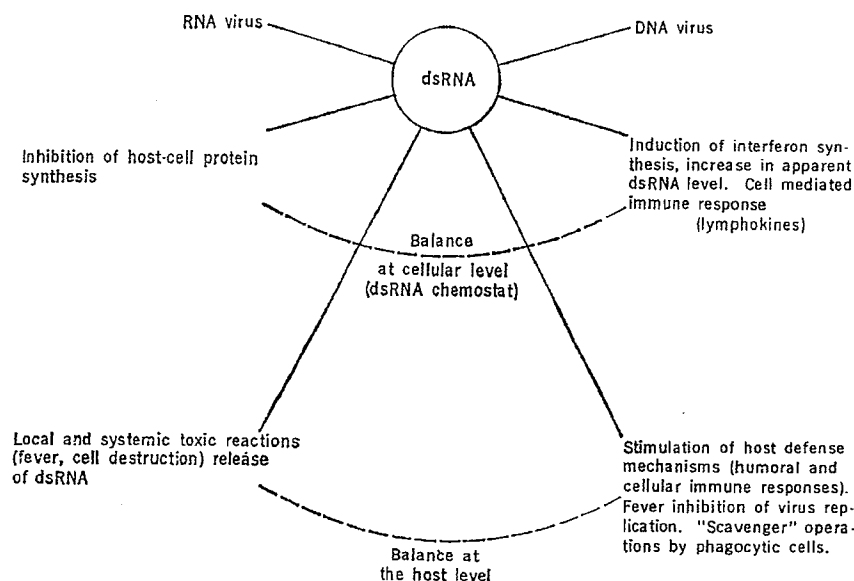


Fig. 1. Modulatory role of dsRNA.

types such as macrophages and granulocytes (58). (The thymus-derived cell appears to be the preferential recipient of dsRNA mediated effects on the immune system.)

4) Fever induction. Many viruses multiply better at temperatures below 37°C, and their multiplication will be inhibited if the body temperature goes above 39°C. Rhinovirus replication, for example, may fall off by 10⁸ log units with a temperature shift of 2°. Carefully controlled body temperature-virus production curves have not been developed at the in vivo level, but temperature increments could exert some modulation effect on replication efficiency. Such increments may not prove to be fine controls, or of relatively great magnitude, under the "typical" naturally occurring infection.

5) Finally, we suggest that the production of dsRNA is undoubtedly detrimental to the virus-infected cell itself, because it accelerates the inevitable onset of cell death. As a direct consequence, the virus-infected cell will be submitted to "scavenger" operations by, for example, phagocytic cells, and thereby a contribution to the final abrogation of the infection will be effected. The scavenger activity should free more double helices into the circulation; additionally, the cell's own dsRNA (as already discussed) would then gain access into biologic fluid and therefore drive up the total dsRNA concentration ultimately available for reactivity for granulocytes, lymphocytes, or other solid tissue.

Conclusion

Double-stranded RNA, made as an intermediary substance in the replication of most, if not all, viruses, may play a much more important role in the pathogenesis and the recovery from virus infections than has hitherto been suspected. Apparently, dsRNA is used by both the challenge virus and the host cell in an attempt to gain "molecular control." Double-stranded RNA exerts a set of effects, which may be well balanced, not only at the level of the individual cell but also at the complex assemblage of these cells termed the organism (Fig. 1). In the cell, interferon synthesis is triggered, although interferon mRNA translation may not occur if dsRNA shuts off protein synthesis too quickly. In the whole organism, the disease severity will depend on how certain toxic reactions evoked

by infection (such as cell necrosis and fever) are counterbalanced by an increase in the host defense mechanisms (for example, immune responsiveness and interferon production). *Many aspects of the response, relating to either progress of, or recovery from, the disease, can be explained on the basis of a dsRNA.*

In addition to drawing attention to the biodynamic role of dsRNA, our hypothesis suggests specific experimental vectors designed to enhance our information on the molecular basis of the morbid process which occurs with viral infection. Finally, we suggest that, although the dsRNA molecule may be viewed as a rather simple unit structure, the opportunity for further diversity in the biological activity of a given dsRNA molecule always exists. Namely, each deviation from a perfectly double-helical arrangement introduces the possibility for emphasizing one biological reactivity at the expense of another. This latter structure-activity property may partially account for the extreme apparent diversity, commonly encountered, in the presentations of virologic illness.

Appendix note added in proof. Subsequent to submission of this text, we have found that the potent mitogen effect of dsRNA for lymphocytes (murine and human) is also exquisitely sensitive to the fidelity in base pairing of the input polymer pair (59). For example, infrequent "loops" (one nucleotide per 20 base pairs) in an otherwise perfectly helical $rL_n \cdot rC_n$ molecule [for example, $rL_n \cdot r(C_{10}U)_n$] strongly changes its mitogenic properties. This observation, which supports our thesis that a "fine structure" term can be developed for other reactions triggered by dsRNA's in biological systems, emphasizes that diverse biological effects may be encountered with an ostensibly uniform family of dsRNA's.

References and Notes

1. W. Jelinek and J. E. Darnell, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2537 (1972).
2. R. Bases and B. H. Kaplan, *Biochim. Biophys. Acta* **312**, 574 (1973).
3. A. R. Hunter, R. T. Hunt, R. J. Jackson, H. D. Robertson, in *Synthese, Struktur und Funktion des Hämoglobins*, H. Martin and L. Nowicki, Eds. (J. C. Lehmanns Verlag, München, Germany, 1972), p. 133.
4. H. D. Robertson and M. B. Mathews, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 225 (1973).
5. A. A. Tytell, G. P. Lampson, A. K. Field, M. R. Nemes, *ibid.* **58**, 1719 (1967).
6. M. M. Nemes, A. A. Tytell, G. P. Lampson, A. K. Field, M. R. Hilleman, *Proc. Soc. Exp. Biol. Med.* **132**, 784 (1969).
7. D. R. Black, F. Eckstein, E. De Clercq, T. C. Merigan, *Antimicrob. Agents Chemother.* **3**, 198 (1973).
8. G. P. Lampson, A. A. Tytell, A. K. Field, M. M. Nemes, M. R. Hilleman, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 782 (1967); G. T. Banks, K. W. Buck, E. B. Chain, F. Himmelweit, J. E. Marks, J. M. Tyler, M. Hollings, F. T. Last, O. M. Stone, *Nature (Lond.)* **218**, 542 (1968).
9. L. F. Ellis and W. J. Kleinschmidt, *Nature (Lond.)* **215**, 649 (1967); W. J. Kleinschmidt, L. F. Ellis, R. M. Van Frank, E. B. Murphy, *ibid.* **220**, 167 (1968); D. N. Planterose, P. J. Birch, D. J. F. Pilch, T. J. Sharpe, *ibid.* **227**, 504 (1970).
10. G. T. Banks, K. W. Buck, E. B. Chain, J. E. Darbyshire, F. Himmelweit, *ibid.* **223**, 155 (1969).
11. P. A. Lemke and T. M. Ness, *J. Virol.* **6**, 813 (1970); K. W. Buck, E. B. Chain, F. Himmelweit, *J. Gen. Virol.* **12**, 131 (1971).
12. G. T. Banks, K. W. Buck, E. B. Chain, J. E. Darbyshire, F. Himmelweit, G. Ratti, T. J. Sharpe, D. N. Planterose, *Nature (Lond.)* **227**, 505 (1970).
13. A. K. Field, G. P. Lampson, A. A. Tytell, M. M. Nemes, M. R. Hilleman, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 2102 (1967).
14. J. Doskocil, N. Fuchsberger, J. Vetrak, V. Lackovic, L. Borecky, *Acta Virol.* **15**, 523 (1971).
15. C. Colby and P. H. Duesberg, *Nature (Lond.)* **222**, 940 (1969); L. M. Montagnier, *Compt. Rend. Acad. Sci. (Paris)* **267**, 1417 (1968); L. Harel and L. Montagnier, *Nat. New Biol.* **229**, 106 (1971); E. De Maeyer, J. De Maeyer-Guignard, L. Montagnier, *ibid.*, p. 109; R. Stern and R. M. Friedman, *Nature (Lond.)* **226**, 612 (1970); P. C. Kimball and P. H. Duesberg, *J. Virol.* **7**, 697 (1971); R. Stern and R. M. Friedman, *Biochemistry* **10**, 3635 (1971).
16. M. H. Lai and W. K. Joklik, *Virology* **51**, 191 (1973); R. Z. Lockart, Jr., N. L. Bayliss, S. T. Toy, F. H. Yin, *J. Virol.* **2**, 962 (1968).
17. J. Simpson, personal communication.
18. R. Falcoff and E. Falcoff, *Biochim. Biophys. Acta* **182**, 501 (1969); *ibid.* **199**, 147 (1970).
19. Abbreviations: U, uridylic acid; G, guanylic acid; I, inosinic acid; A, adenylic acid; r(C)_n, r(D)_n, etc., polynucleotides of repeating sequence for C, I, etc.; a subscript other than n denotes the number of units in the polymer; IgA, IgG, IgM, immunoglobulins A, G, and M.
20. E. De Clercq, in *Topics in Current Chemistry*, F. L. Boschke, Ed. (Springer-Verlag, New York, in press).
21. J. Vilcek, M. H. Ng, A. E. Friedman-Kien, T. Krawciw, *J. Virol.* **2**, 648 (1968); C. Colby and M. J. Chamberlin, *Proc. Natl. Acad. Sci. U.S.A.* **63**, 160 (1969); C. Colby, B. D. Stollar, M. I. Simon, *Nat. New Biol.* **229**, 172 (1971).
22. S. Baron, N. N. Bogomolova, A. Billiau, H. B. Levy, C. E. Buckler, R. Stern, R. Naylor, *Proc. Natl. Acad. Sci. U.S.A.* **64**, 67 (1969); A. Billiau, C. E. Buckler, F. Dianzani, C. Unlendorf, S. Baron, *Proc. Soc. Exp. Biol. Med.* **132**, 790 (1969); P. M. Pitha and J. Pitha, *J. Gen. Virol.*, in press.
23. W. A. Carter, P. M. Pitha, L. W. Marshall, I. Tazawa, S. Tazawa, P. O. P. Ts'o, *J. Mol. Biol.* **70**, 567 (1972).
24. T. Hunt and E. Ehrenfeld, *Nat. New Biol.* **230**, 91 (1971); E. Ehrenfeld and T. Hunt, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1075 (1971); J. Chao, L. Chao, J. Speyer, *Biochem. Biophys. Res. Commun.* **45**, 1096 (1971).
25. R. Kaempfer and J. Kaufman, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1222 (1973).
26. B. Cordell-Stewart and M. W. Taylor, *J. Virol.* **11**, 232 (1973).
27. ———, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1326 (1971).
28. M. H. Vodkin and G. R. Fink, *ibid.* **70**, 1069 (1973).
29. M. W. Taylor, B. Cordell, M. Souhrada, S. Prather, *ibid.* **68**, 836 (1971).
30. F. T. Serota and R. Baserga, *Science* **167**, 1379 (1970); R. I. Jahiel, D. Taylor, N. Rainford, S. E. Hirschberg, R. Kroman, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 740 (1971).
31. M. Absher and W. R. Stinebring, *Nature (Lond.)* **223**, 715 (1969); H. L. Lindsay, P. W. Town, J. Brandt, M. Forbes, *ibid.*, p. 717; R. H. Adamson and S. Fabro, *ibid.*, p. 718; H. B. Oster, J. O. Oh, C. R. Dawson, W. L. Burt, *ibid.* **228**, 362 (1970).
32. P. Jullien and J. De Maeyer-Guignard, *Int. J. Cancer* **7**, 468 (1971); F. S. Philips, M. Fleisher, L. D. Hamilton, M. K. Schwartz, S. S. Sternberg, in *Biological Effects of Polynucleotides*, R. F. Beers, Jr., and W. Braun,

- Eds. (Springer-Verlag, New York, 1971), p. 259; M. Degre, *Proc. Soc. Exp. Biol. Med.* 142, 1087 (1972); B. J. Leonard, E. Eccleston, D. Jones, *Nature (Lond.)* 224, 1023 (1969).
33. B. J. Leonard and E. Eccleston, *Proceedings of the European Society for the Study of Drug Toxicity* 13, 274 (1972); A. D. Steinberg, S. Baron, N. Talal, *Proc. Natl. Acad. Sci. U.S.A.* 63, 1102 (1969).
 34. P. A. Young, J. J. Taylor, M. C. Yu, E. Eyerman, *Nature (Lond.)* 228, 1191 (1970); M. C. Yu, P. A. Young, W. A. Yu, *Am. J. Pathol.* 64, 305 (1971).
 35. A. K. Field, C. W. Young, I. H. Krakoff, A. A. Tytell, G. P. Lampson, M. M. Nemes, M. R. Hilleman, *Proc. Soc. Exp. Biol. Med.* 136, 1180 (1971); D. A. Hill, S. Baron, H. B. Levy, J. Bellanti, C. E. Buckley, G. Cannellos, P. Carbone, R. M. Chanock, V. DeVita, M. A. Guggenheim, E. Homan, A. Z. Kapikian, R. L. Krischstein, J. Mills, J. C. Perkins, J. E. Van Kirk, M. Worthington, in *Perspectives in Virology*, M. Pollard, Ed. (Academic Press, New York, 1971), p. 197.
 36. J. Vilcek and M. H. Ng, *Advan. Protein Chem.*, in press.
 37. W. A. Carter, L. Marshall, P. O. P. Ts'o, unpublished observations.
 38. P. M. Pitha, L. W. Marshall, W. A. Carter, *J. Gen. Virol.* 15, 89 (1972); E. De Clercq, R. D. Wells, R. C. Grant, T. C. Merigan, *J. Mol. Biol.* 56, 83 (1971).
 39. K. Amako and S. Dales, *Virology* 32, 201 (1967).
 40. A. T. Haase, S. Baron, H. Levy, J. A. Kasel, *J. Virol.* 4, 490 (1969).
 41. H. B. Levy and W. A. Carter, *J. Mol. Biol.* 31, 561 (1968).
 42. R. K. Ralph, *Advan. Virus Res.* 15, 61 (1969).
 43. P. H. Duesberg and C. Colby, *Proc. Natl. Acad. Sci. U.S.A.* 64, 396 (1969); C. Colby, C. Jurale, J. R. Kates, *J. Virol.* 7, 71 (1971).
 44. J. J. Lucas and H. S. Ginsberg, *Biochem. Biophys. Res. Commun.* 49, 39 (1972).
 45. R. M. Franklin, *Proc. Natl. Acad. Sci. U.S.A.* 55, 1504 (1966).
 46. W. E. Stewart II, E. De Clercq, A. Billiau, J. Desmyter, P. De Somer, *ibid.* 69, 1861 (1972).
 47. E. De Clercq, W. E. Stewart II, P. De Somer, *Infect. Immunol.* 7, 167 (1973).
 48. W. E. Stewart II, E. De Clercq, P. De Somer, *J. Gen. Virol.* 18, 237 (1973).
 49. W. Braun and M. Nakano, *Science* 157, 819 (1967); R. Winchurch and W. Braun, *Nature (Lond.)* 223, 843 (1969); A. F. Woodflour, A. Friedman, A. A. Tytell, M. R. Hilleman, *Proc. Soc. Exp. Biol. Med.* 131, 809 (1969); W. Turner, S. P. Chan, M. A. Chirigos, *ibid.* 133, 334 (1970); H. Cantor, R. Asofsky, H. B. Levy, *J. Immunol.* 104, 1035 (1970); J. R. Schmidke and A. G. Johnson, *ibid.* 106, 1191 (1971); T. J. Chester, E. De Clercq, T. C. Merigan, *Infect. Immun.* 3, 516 (1971); D. Collavo, B. Finco, L. Chieco-Blanchi, *Nat. New Biol.* 239, 154 (1972).
 50. H. M. Friedman, A. G. Johnson, P. Pan, *Proc. Soc. Exp. Biol. Med.* 132, 916 (1969); J. H. Dean, W. C. Wallen, D. O. Lucas, *Nat. New Biol.* 237, 218 (1972); T. A. McNeill, *Immunology* 21, 741 (1971).
 51. D. Waddell, T. C. Merigan, J. Wilbur, S. Walker, *Clin. Res.* 5, 312 (1967).
 52. R. E. Cone and A. G. Johnson, *J. Exp. Med.* 133, 665 (1971); *Cell Immunol.* 3, 283 (1972); B. N. Jaroslow and L. Ortiz-Ortiz, *ibid.* 3, 123 (1972).
 53. K. Y. Huang, R. M. Donahue, F. B. Gordon, H. B. Dressler, *Infect. Immunol.* 4, 581 (1971).
 54. E. De Clercq and W. E. Stewart II, in *Selective Inhibitors of Viral Functions*, W. A. Carter, Ed. (Chemical Rubber Co., Cleveland, 1973), p. 81.
 55. W. E. Stewart II, in *Selective Inhibitors of Viral Functions*, W. A. Carter, Ed. (Chemical Rubber Co., Cleveland, 1973), p. 1.
 56. D. C. Dumonde, R. A. Wolstencroft, G. S. Panayi, M. Mathew, J. Morley, W. T. Howson, *Nature (Lond.)* 224, 38 (1969).
 57. M. C. Raff, *ibid.* 242, 19 (1973).
 58. G. A. Granger, *Ser. Haematol.* 5, 8 (1972).
 59. W. A. Carter, J. S. Horoszewicz, P. O. P. Ts'o, in preparation.
 60. D. Baltimore, *Bacteriol. Rev.* 35, 235 (1971); *Trans. N.Y. Acad. Sci.* 173, 327 (1971); W. H. Mitchell, in *Selective Inhibitors of Viral Functions*, W. A. Carter, Ed. (Chemical Rubber Co., Cleveland, 1973), p. 60.
 61. We happily acknowledge the insight of Dr. Eugene Suikowski whose comments sharpened the definition of a number of our ideas. Professor P. De Somer and Drs. A. Bardbournie and W. E. Stewart II also gave constructive review. The research of W.A.C. cited here was supported by NIH grant A1-11292-07; by a Center grant in Viral Chemotherapy (CO 14801-01); by the Jane Coffin Childs Fund for Medical Research (286); and, in part, by contract NO1-CM33726 with the Chemotherapy Branch, National Cancer Institute. E.DeC. was supported by a grant from the Belgian Fonds voor Geneeskundig Wetenschappelyk Onderzoek.

The ATS-1 Medical Network

Since 1971, the Advanced Technology Satellite (ATS-1) launched by the National Aeronautics and Space Administration (NASA) has been used in a program for delivering health care to rural populations in Alaska (1). The Tanana Service Unit in central Alaska an area about the size of Texas, was chosen as the first experimental site because of the nature of the terrain and climate and because the Indian Health Service, a sister organization at HEW, has responsibility for the well-being of all Alaskan Indians. The majority of the native population is scattered in some 200 villages over the length and breadth of the state. Seven health service units, each with a service unit hospital, serve these villages. The major hospital to which patients are referred is located in Anchorage. Primary health care in the villages is administered by a community health aide who has received up to 16 weeks of training by the Public Health Service (PHS). The health aide's tools are a basic drug kit, a manual, and a high-frequency (hf) radio that may be used to contact a PHS physician on a daily schedule and in times of emergency. The hf radio is plagued by ionospheric interference that causes periods of "blackout" (no communications) which

Health Care and Education: On the Threshold of Space

Audio and video satellite communications are being used experimentally for health care and education in Alaska.

Albert Feiner

It is not universally agreed that there is an absolute shortage of physicians, but it is so agreed that there exists a maldistribution of medical services that leaves many millions of Americans with minimal or no primary health care. The problem must be attacked from two directions if the situation is to be alleviated: physicians must be trained so that their undergraduate and postgraduate experiences will be rooted

in rural America, and acceptable substitutes must be found for the physical presence of highly qualified physicians and teachers of medicine. The Department of Health, Education, and Welfare (HEW) has examined both approaches to the problem and recently, at the Lister Hill National Center for Biomedical Communications, a part of the National Library of Medicine, scientists have been exploring the possibility of using advanced telecommunications techniques to deliver health care and medical education to populations where these commodities are scarce.

Mr. Feiner is director of technology evaluation and management at Practical Concepts Inc., 1030 15th Street, NW, Washington, D.C. 20005. He was formerly director of the Lister Hill National Center for Biomedical Communications, National Library of Medicine, Bethesda, Maryland.